CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
TCMTB
[2-(thiocyanomethylthio)benzothiazole]

Chemical Code # 971, Tolerance # 288
SB 950 # 116

February 22, 2001
Revised February 8, 2002, August 12, 2002 and March 17, 2004

I. DATA GAP STATUS

Combined, rat:	No data gap, possible adverse effect indicated (0nco)
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect.
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 187924 in 288 - 072 were examined.
** Indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T040317
Toxicology Summary by Kishiyama and Gee, 2/22/01, revised 2/8/02, 8/12/02 and 3/17/04 [correction, no new data]

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

288 - 071 187923 "Review of the stability of TCMTB as used in toxicology studies conducted at Inveresk Research , Inveresk Project No. 342355." (N, B. Dinwoodie, Inveresk Research, 6/28/02, for Buckman Laboratories) This brief report addresses the issue of the stability of batch 1517 lot 6-14285, which was used in the combined rat, the chronic dog and the mouse oncogenicity studies, all conducted at Inveresk in the late 1980's. All three were considered unacceptable based on the uncertainty of the stability of the TCMTB over the testing periods. Record 187923 contains chromatograms of TCMTB from September 8, 1987 and January 12, 1990, performed at Inveresk. The text discusses the breakdown products, etc., expected if there was significant degradation. From a comparison of the two profiles, there does not appear to be any major changes. Therefore, the three studies in question, record numbers 114111, 114113 and 114114 have been upgraded to acceptable status. No worksheet. One-liners have been updated to reflect changes with no supplemental worksheets. (Gee, 8/12/02).

COMBINED, RAT

**** 037, 071 114111, 187923** Everett, D. J., C. J. Perry, C. Atkinson and J. Finch. "2-(Thiocyanomethylthio) Benzothiazole (TCMTB) 104 Week Dietary Carcinogenicity Study in Rats with 52 Week Interim Kill (Results after 104 Weeks)". (Inveresk Research International, IRI Project No. 435313, Report No. 7351, September 11, 1989.) TCMTB (lot 6-14285, 81.6%) was fed in the diet at doses of 0, 2, 8 or 20 mg/kg b. wt./day for 52 weeks (interim group) or 104 weeks (carcinogenicity group) to 20 (interim) or 50 (carcinogenicity) Sprague-Dawley rats/sex/group. At Week 52, slight increases in liver weight and increased mitoses in liver sections (3/20) were noted for high-dose females but not for males. These findings were not noted in the terminal sacrifice animals. At 20 mg/kg, there was parakeratosis of the stomach of males (23/50, $p < 0.01$) and females (21/50, $p < 0.01$). The authors suggested this may have been due to irritation of the test material. Systemic NOEL = 8 mg/kg/day (stomach parakeratosis). At Week 104, increased incidences of benign testicular interstitial cell tumors were found at 8 mg/kg (13/50) and at 20 mg/kg (14/50), both statistically significantly increased over control incidence (4/50) at $p < 0.05$. No other treatment related effects were reported. Evaluated as unacceptable but upgradeable (stability of the technical test article was not verified for the length of the study). **Possible adverse effect** (testicular interstitial cell tumors). (Kishiyama and Gee, 2/15/01) Record 187923 addressed the stability issue, upgrading the study to ACCEPTABLE. (Gee, 8/12/02).

288-038 114112 Interim, 52-week report for 114111 in 288-037 above. Not reviewed. No worksheet. (Gee, 2/21/01).

288-062 183388 "Establishment of methodology for the analysis of TCMTB in diets; the assessment of diet mixing procedures and the stability of TCMTB in such diets. The routine analysis of dietary formulations prepared for a 104 week carcinogenicity study in rats (IRI Project No. 435313) and a 104 week carcinogenicity study in mice (IRI Project No. 435329)" and "The routine analysis of dietary formulations prepared for a 2 generation reproduction study in rats (IRI Project No. 435334) and a 52 week toxicity study in dogs (IRI Project No. 635606)."

Dinwoodie, N. B. (Inveresk Research International, IRI Projects Nos. 335306 and 335290, Report No. 8041, 10/3/91) This document was sent in response to earlier reviews of the studies in the rat and mouse in particular. The review worksheets requested data on the stability of TCMTB technical over the life of the studies. The document supplied contained duplicate pages for 1122 to 1133 in the original report, 288-037, Record 114111. (Gee, 2/6/02)

288 - 064 183390 Atkinson, C., C. J. Perry and J. Finch " 2-(Thiocyanomethylthio) benzothiazole (TCMTB) 13 week dietary dose range finding study in rats." (Inveresk Research International, Scotland, IRI project 435528, report 3930, June 12, 1987) Groups of 10 Sprague-Dawley rats /sex were fed diets containing TCMTB (lot 6-142-85, batch 1517, 81.5%) at concentrations adjusted to give doses of 0, 10, 30, 70 or 100 mg/kg/day over 13 weeks. Limited parameters were measured including body weight, food consumption, organ weights and stomach tissues. No hematology, clinical chemistry, urinalysis or ophthalmology was included. Limited tissues were examined at necropsy. Body weight and weight gain were lower at 70 and 100 mg/kg/day, food consumption was also lower at these doses in males and at 100 mg/kg in females. Stomach histopathology was not examined at 100 mg/kg/day but squamous epithelial hyperplasia was seen at 30 and 70 mg/kg with a dose response in incidence and severity. NOEL = 10 mg/kg/day nominal dose (stomach pathology, body weight, food consumption). Supplemental range-finding study. (Gee, 2/6/02).

288-015 015936 “90-Day subchronic oral toxicity study of TCMTB in rats.” (Rao, G. N., Director of Toxicology, Raltech Scientific Services, Study No. 79003, 2/22/80) TCMTB (lot no 9-3573, no purity stated) was fed in the diet to Sprague-Dawley derived rats at 0, 333, 500 or 750 ppm, 30/sex/group, for 13 weeks. Ten/sex/group were selected for an interim sacrifice at 45 days. There were no effects on clinical signs, hematology, clinical chemistry, urinalysis, or organ weights. Ophthalmology was not done. The only effects reported were on the stomach. This consisted of inflammatory cell infiltration in 4/20 high dose males and 7/20 high dose females ($p < 0.01$) with 0/19 and 0/20 in controls for males and females. In addition, females also showed inflammation (4/20 at 750 ppm), edema (2/19 at 500 and 7/20 at 750 ppm), degeneration or necrosis of squamous epithelium (5/20 at 750 ppm ($p < 0.05$)) and ulcer formation (3/20 at 750 ppm). The NOEL based on the limited effects was 333 ppm for females and 500 ppm for males. The study was UNACCEPTABLE, based on an inadequate high dose level, lack of ophthalmology and lack of purity of the test material and data on intake of TCMTB over the study. Not upgradeable. (Gee, 2/15/01) Note: This study was reviewed to determine if it supported the dose selection in the 104 week study, record no. 114111.

CHRONIC TOXICITY, DOG

** 039, 063, 071 114113, 183389, 187923 Goburdhun, R. and R J. Greenough. “2-(Thiocyanomethylthio) Benzothiazole (TCMTB) 52 Week Dietary Toxicity Study in Dogs”. (Inveresk Research International, IRI Project No. 635605, Report No. 5646, November 21, 1988.) TCMTB (lot 6-14285, 81.6%) was admixed with the feed at concentrations of 0, 100, 300 and 1000 ppm and fed for 52 consecutive weeks to 4 Beagle dogs/sex/group. Achieved doses were 3.8, 11.7 and 38.8 mg/kg/day for males and 4.0, 11.2 and 43.2 mg/kg/day for females. There were no significant findings for clinical signs, hematology, ophthalmology, urinalysis, organ weights or histology. Alanine aminotransferase and total bilirubin values were lower in the treated

groups of males and females with the reduction having some dose relationship. The toxicological

meaning of these values, however, in the absence of other findings, was considered questionable. NOEL = 300 ppm (lower body weights). Unacceptable. Upgradeable (justification of the dose selection, based on palatability [IRI 3729], and stability data for lot 6-14285). (Kishiyama and Gee, 2/20/01) Record 183389 (see below) was submitted in response to the review of this study. The stability of the test article over the life of the study is still unanswered. (Gee, 2/6/02). Record 187923 addressed the stability issue. ACCEPTABLE. (Gee, 8/12/02)

288 - 063 183389 Greenough, R. J. and R. Goburdhun "TCMTB: Dietary palatability study in dogs." (Inveresk Research International Limited, IRI Project 635893, report no. 3729, 12/23/86) [Supplemental to record no 114113] Beagle dogs, 2/sex/dose group, were fed diets containing 0, 750, 1000 or 1500 ppm TCMTB (lot 6-14285, batch 1517) for 14 consecutive days. Each dog was offered 400 g of food/day in the morning with the food residue measured the following day. Water was available *ad libitum*. Fresh diet was prepared weekly. Body weights, food consumption and clinical signs were recorded. At necropsy, the liver, heart, kidneys and spleen were weighed. There were no clinical signs. Body weight losses were seen at 1500 ppm in both sexes (males: -0.8 and -0.2 kg, females: -0.2 kg for both animals). Food consumption at 1500 ppm was approximately 50% of the pretrial intake. Food intake was moderately lower in females at 1000 ppm. The conclusion of the investigators was that a concentration <1500 ppm should be used for a long-term feeding study. This report justifies the doses used in the 1-year study in record 114113. No worksheet. (Gee, 2/6/02).

ONCOGENICITY, RAT

See COMBINED, RAT.

ONCOGENICITY, MOUSE

** 040, 071 114114, 187923 Everett, D. J., C. Atkinson, J. Heath, and W. Henderson. "2-(Thiocyanomethylthio) Benzothiazole (TCMTB) 104 Week Dietary Carcinogenicity Study in Mice". (Inveresk Research International, IRI Project No. 435329, Report No. 7516, 1/24/90). TCMTB (lot 6-14285, 81.6%) was admixed with the feed at doses of 0, 15, 50 or 150 mg/kg- b. wt./day and fed for 104 weeks to 50 CD-1 mice/sex/group. There was reduced body weight and bodyweight gain in both sexes at the high dose (150 mg/kg). Also, an increase in the incidence of focal and diffuse hyperplasia of the duodenal mucosa was reported for high dose males (10/50 versus 1/50 in controls). Nominal systemic NOEL = 50 mg/kg/day. There was no evidence of an oncogenic effect at the doses tested. Unacceptable (dose selection needs justification). Possibly upgradeable with submission of the 13-week study and stability data (see worksheet). (Kishiyama and Gee 2/16/01). See 183391 for the 13-week study. (Gee, 2/7/02) Record 187923 addressed the stability issue. ACCEPTABLE. (Gee, 8/12/02)

288 - 065 183391 Atkinson, C. and C. J. Perry "2-(Thiocyanomethylthio) benzothiazole (TCMTB) 13 week dietary dose range finding study in mice." (Inveresk Research International, IRI 435381, Report No. 3923, June 19, 1987) TCMTB (81.6%, lot 6-14285) was fed in the diet at concentrations to give doses of 0, 10, 30, 70 or 100 mg/kg/day to 10 CD-1 mice/sex/group for 13 weeks. The study was designed as a range-finding study so only limited parameters were examined. These included body weight, food consumption, organ weights and limited tissues for histopathology for the control and high dose groups. There was a slightly lower food intake in males at 70 and 100 mg/kg and in females at 100 mg/kg/day. There were no dose-related effects

on body weights except that weight and weight gain in all treated male groups were lower than controls. This was not seen in females, which were comparable with controls. Organ weights and histological findings were comparable with controls. No NOEL was determined from the study. Supplemental study. (Gee, 2/7/02)

REPRODUCTION, RAT

** 288 - 072 187924 " TCMTB two generation reproduction study in rats." (K. P. Hazelden and J. A. Wilson, Inveresk Research International, Report No. 5168, Project No. 435334, 11/22/88). TCMTB (batch 1517, presumed 81.6%) was fed to 24/sex/group of Sprague-Dawley rats at 0, 25, 100 or 400 ppm for two generations, 1 litter in the first and 2 litters in the second. The second mating of the F1 adults was performed because of the lower fertility in all groups in the first mating. There were no affects on body weight, food consumption, clinical signs or reproductive parameters in either generation. The only finding was a statistically significant lower pup weight at 400 ppm in the second mating (F2b pups), being approximately 88% of control pup weight. Although not significant, the mean weight of these pups was also lower at 7 and 14 days. Although pup weights were lower in the F2a generation at 400 ppm, the difference was not statistically significant. Parental NOEL = 400 ppm (no affects); Pup NOEL = 100 ppm (based on lower F2b pup weight only); reproductive NOEL = 400 ppm (no affects). ACCEPTABLE. (Gee, 8/12/02)

TERATOLOGY, RAT

** 020, 066 038254, 183392 "Teratogenicity Study of TCMTB in Rats Project BUC-TR-001" (Phillip T. Goad, Intox Laboratories, Arkansas, 9/19/85) TCMTB, Lot 511230, Batch 1381, no purity given, was administered to presumed pregnant Crl:CD®(SD)BR rats by gavage on days 6-15 of gestation at 0 (corn oil with 1% Tween 80), 25.1, 76.5, or 125.5 mg/kg, 29 rats/group. Analyses of the dosing material for content, homogeneity and stability were included. Ethylene glycol monoethyl ether at 200 mg/kg was used as a positive control and administered by gavage on days 10-12 of gestation. Dose related signs of clinical toxicity (rough hair coat, diarrhea/loose stool, urine staining and others) were observed at all dose levels. There was a modest but not statistically significant decrease in pregnancy weight gain at the high dose level. NOEL (maternal) < 25.1 mg/kg (clinical signs). Developmental NOEL = 125.5 mg/kg (HDT). No adverse effect. Unacceptable, but upgradeable with purity of the test article. (Gee, 2/21/01) Record 183392 contains the purity of the test article lot as 83.6%, upgrading the study to ACCEPTABLE status. (Gee, 2/7/02)

288 - 066 183392 Two page document containing the analysis of lot 5-11230, giving TCMTB content as 83.6%. This information was requested for record 038254 and upgrades that study to acceptable status. (Gee, 2/7/02).

TERATOLOGY, RABBIT

** 027, 067 063578, 183393 "A Teratology Study in Rabbits with TCMTB" (Adam, G. P.,

WIL Research Laboratories, Project No. WIL-94014, 10/10/86) TCMTB, Lot No. 5-13002, 81.0%, purity, was administered to artificially inseminated New Zealand White rabbits by gavage on days 6-19 of gestation at nominal doses of 0 (corn oil with 1% Tween-80), 10, 20 or 40 mg/kg, 20 rabbits/group. NOEL (maternal) = 20 mg/kg. At 40 mg/kg, there was a significant ($p<0.01$) reduction in body weight gain for days 6-12 and days 6-19. Body weight gain was greater than control ($p<0.01$) for days 19-29, after the cessation of treatment. Food consumption pattern was similar to weight gain for the same time intervals. Effects at 20 mg/kg were very slight and not statistically significant. Following cessation of treatment, body weight gain and food consumption were statistically greater than controls. Developmental NOEL = 40 mg/kg (HDT). No fetal effects were seen. No adverse effects were reported. Unacceptable but upgradeable with information on dosing solution stability, preparation and a range-finding study to justify the dose levels. (Gee, 2/20/01). The range-finding study was submitted in 288 - 067, 183393, and some analytical data for dosing analysis were included in Appendix of that volume. The study is upgraded with the supplemental data. (Gee, 2/8/02).

288 - 067 183393 Adam, G. P. " A range-finding teratology study in rabbits with TCMTB." (WIL Laboratories, project WIL-94013, 10/10/86) TCMTB (81%, lot 5-13002) was given by gavage to 5 New Zealand White rabbits per dose at 0, 25, 50, 75, 150 or 300 mg/kg/day, days 6 through 19, in the initial study. Three other groups were added when excessive toxicity was seen the higher doses. These were given 10, 25 or 40 mg/kg/day with no additional vehicle controls. All does died at 150 and 300 mg/kg/day with 3 deaths at 50 and 75 mg/kg/day. No affect on body weight was seen at 10 mg/kg/day. No apparent effect on intrauterine survival was noted. Fetuses were not evaluated. From the results of this range-finding study, 40 mg/kg/day was selected as the high dose in the definitive study. [This volume contained Appendices A and B, analytical data and historical control data, which were no included in the original submission, volume 027, record 063578.] Supplemental study. (Gee, 2/8/02).

288 - 068 183394 The document contained a duplicate of Appendix A from the range-finding study, 183393, which contained analyses of the dosing material from 94014 on two days of dosing, the first and last, with adequate test article content. Duplicate of Appendix A in 067 183393. (Gee, 2/8/02).

GENE MUTATION

** 008 952367 "Determination of the Mutagenic Potential of TCMTB Using the CHO/HGPRT Chinese Hamster Ovary Cell Forward Mutation Assay". (Raltech Scientific Services Inc., 12/1/80) TCMTB (no identification or purity given) was assayed with CHO cells with activation at 7 concentrations ranging from 1 to 15 μg/ml, a repeat trial was conducted at 7, 8, 9, 10,12 and 12 μg/ml to determine if one elevated value was a true value. Without activation, test

concentrations were 0.1, 0.5, 0.75, 1./0 and 1.5 μg/ml. Each dose was tested in duplicate. Cytotoxicity data was not shown, nor was survivability in the expression cultures. No evidence of mutation was present. Acceptable**.** **(**J. Christopher, 4/18/85.)

CHROMOSOME EFFECTS

** 022 046227 "Clastogenic Evaluation of TCMTB, Lot 5-13002, In The *in vivo* Mouse

Micronucleus Assay". (James L. Ivett, Hazleton Biotechnologies Co., Project No. 20996, 6/86). TCMTB, Lot 5-13002, 81%, density of 1.38, was given to male and female ICR mice in a single dose by gavage at 0 (corn oil), 50, 167 or 500 mg/kg. Animals in the treated groups were sacrificed at 24, 48 and 72 hours after treatment, 5/sex/group/time point. Doses were adjusted for density but not for purity. Negative controls (corn oil) and positive controls, TEM at 1.5 mg/kg by i.p. injection, were sacrificed at 24 hours only. At 500 mg/kg, animals appeared lethargic with rapid, shallow breathing immediately after dosing. At 50 and 167 mg/kg, animals had scruffy coats. There were 6 mortalities at 500 mg/kg over the study. Bone marrow from the tibias was removed and analyzed for the presence of micronuclei in polychromatic erythrocytes and for PCE:RBC. Analyzed 1000 PCE per animal. There was no increase in micronuclei in the PCEs of treated animals. The ratio of PCE:RBC was lower at the high dose in both sexes. The positive control was functional. ACCEPTABLE with the deficiency of negative control data at 24 hours only. (Gee, 2/21/01)

027 63576 Exact duplicate of 022 46227.

DNA DAMAGE

** 008 952368 "Determination of Mutagenic Potential of TCMTB Using *in vitro* Chinese Hamster Ovary Cell Sister Chromatid Exchange Assay" (Raltech Scientific Services Inc., 12/8/80) TCMTB (no characterization given) was assayed for sister chromatid exchange with CHO cells at concentrations of 0.75, 1.0, 2.5, 5.0 and 10.0 µg/ml with metabolic activation. Concentrations of 0.05, 0.75, 0.1, 0.5 and 1.0 µg/ml were tested in the absence of metabolic

activation. All exposure was for 4 hours, assays with single cultures. There was no evidence of induction of sister chromatid exchanges. **Acceptable.** This study was considered acceptable although the test article was not characterized, use of single cultures only, and no cytotoxicity data presented. (J. Christopher, 4/18/85.)

** 027 063496 "Evaluation of TCMTB, Lot No. 5-13002, in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay". (Maria A. Cifone, Hazleton Biotechnologies Co., Project No. 20991, 8/86) TCMTB, Lot No. 5-13002, 81%, was assayed for unscheduled DNA synthesis with monolayer cultures of rat hepatocytes. The test was conducted at 12 concentrations from 50 to 0.01 µg/ml. The 50 and 25 µg/ml were extremely toxic levels and not suitable for analysis. The assay was scored at 8 concentrations ranging from 10.0 to 0.05 µg/ml, which had 38.4 % to 99.3% survival, respectively. Each treatment level had 5 cultures, 2 of which were used for cytotoxicity by trypan blue dye exclusion. Exposure was for 18-19 hours. Positive control was 2-acetylaminofluorene at 0.05 or 0.10 µg/ml. The net nuclear grain count was scored for 50

cells/coverslip. There was no increase in UDS in treated cells**.** Unacceptable but upgradeable with submission of additional data regarding individual cultures. (Gee, 2/20/01) With record 183395, the study has been upgraded to ACCEPTABLE status. (Gee, 2/8/02)

288 - 069 183395 "Evaluation of TCMTB, Lot No. 5-13002, in the Rat Primary Hepatocyte

Unscheduled DNA Synthesis Assay." Supplement to 063495 containing the individual slide data for mean net nuclear grains, mean cytoplasmic grains and % cells with >6 NNG. These data were requested for upgrading the study. Although not ideal, these additional data improved the ability

to evaluate the study as having no indication of UDS induction. (Gee, 2/8/02).

NEUROTOXICITY

Not required at this time